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(54) Title: 6-SUBSTITUTED PYRAZOLO [3,4-d] PYRIMIDIN-4-ONES USEFUL AS CYCLIN DEPENDENT KINASE INHIBITORS

(57) Abstract: This invention relates to 6-substituted pyrazolo[3,4-d]pyrimidin-4-ones useful as cyclin dependent kinase (cdk) inhibitors, pharmaceutical compositions comprising the same and methods for using these compounds for treating cancer and proliferative diseases.

TITLE

6-Substituted pyrazolo[3,4-d]pyrimidin-4-ones Useful as Cyclin Dependent Kinase Inhibitors

FIELD OF THE INVENTION

This invention relates to 6-substituted pyrazolo[3,4-d]pyrimidin-4-ones useful as cyclin dependent kinase (cdk) inhibitors, pharmaceutical compositions comprising the same, methods for using these compounds for treating cancer and proliferative diseases, and intermediates and processes for making the same.

BACKGROUND OF THE INVENTION

One of the most important and fundamental processes in biology is the division of cells mediated by the cell cycle. This process ensures the controlled production of subsequent generations of cells with defined biological function. It is a highly regulated phenomenon and responds to a diverse set of cellular signals both within the cell and from external sources. A complex network of tumor promoting and suppressing gene products are key components of this cellular signaling process. Overexpression of the tumor promoting components or the subsequent loss of the tumor suppressing products will lead to unregulated cellular proliferation and the generation of tumors (Pardee, Science 246:603-608, 1989).

Cyclin dependent kinases play a key role in regulating the cell cycle machinery. These complexes consist of two components: a catalytic subunit (the kinase) and a regulatory subunit (the cyclin). To date, eight kinase subunits (cyclin dependent kinase 1-8) have been identified along with several regulatory subunits (cyclins A-H, K, N, and T). Each kinase associates with a specific regulatory partner and together make up the active catalytic moiety. Each transition of the cell cycle is regulated by a particular cyclin dependent

kinase complex: G1/S by cyclin dependent kinase2/cyclin E, cyclin dependent kinase4/cyclin D1 and cyclin dependent kinase6/cyclinD2; S/G2 by cyclin dependent kinase2/cyclin A and cyclin dependent kinase1/cyclin A; G2/M by cyclin dependent kinase1/cyclinB. The coordinated activity of these kinases guides the individual cells through the replication process and ensures the vitality of each subsequent generation (Sherr, Cell 73:1059-1065, 1993; Draetta, Trends Biochem. Sci. 15:378-382, 1990).

An increasing body of evidence has shown a link between tumor development and cyclin dependent kinase related malfunctions. Over expression of the cyclin regulatory proteins and subsequent kinase hyperactivity have been linked to several types of cancers (Jiang, Proc. Natl. Acad. Sci. USA 90:9026-9030, 1993; Wang, Nature 343:555-557, 1990). More recently, endogenous, highly specific protein inhibitors of cyclin dependent kinases were found to have a major affect on cellular proliferation (Kamb et al., Science 264:436-440, 1994; Beach, Nature 336:701-704, 1993). These inhibitors include $p16^{INK4}$ (an inhibitor of cyclin dependent kinase4/D1), $p21^{CIP1}$ (a general cyclin dependent kinase inhibitor), and $p27^{KIP1}$ (a specific cyclin dependent kinase2/E inhibitor). A recent crystal structure of p27 bound to cyclin dependent kinase2/A revealed how these proteins effectively inhibit the kinase activity through multiple interactions with the cyclin dependent kinase complex (Pavletich, Nature 382:325-331, 1996). These proteins help to regulate the cell cycle through specific interactions with their corresponding cyclin dependent kinase complexes. Cells deficient in these inhibitors are prone to unregulated growth and tumor formation.

Protein kinases, in particular, cdk, play a role in the regulation of cellular proliferation. Therefore, cdk inhibitors can be useful in the treatment of cell proliferative disorders such as cancer, familial

adenomatosis polyposis, neuro-fibromatosis, psoriasis, fungal infections, endotoxic shock, trasplantaion rejection, vascular smooth cell proliferation associated with atherosclerosis, pulmonary fibrosis, arthritis glomerulonephritis and post-surgical stenosis and restenosis (U.S. Patent No. 6,114,365). Cdks are also known to play a role in apoptosis. Therefore cdk inhibitors, could be useful in the treatment of cancer; viral infections, for example, herpevirus, poxvirus, Epstein-Barr virus, Sindbis virus and adenovirus; prevention of AIDS development in HIV-infected individuals; autoimmune diseases, for example, systemic lupus, erythematosus, autoimmune mediated glomerulonephritis, rheumatoid arthritis, psoriasis, inflammatory bowel disease, and autoimmune diabetes mellitus; neurodegenerative disorders, for example, Alzheimer's disease, AIDS-related dementia, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, spinal muscular atrophy and cerebellar degeneration; myelodysplastic syndromes, aplastic anemia, ischemic injury associated with myocardial infarctions, stroke and reperfusion injury, arrhythmia, atherosclerosis, toxin-induced or alcohol related liver diseases, hematological diseases, for example, chronic anemia and aplastic anemia; degenerative diseases of the musculoskeletal system, for example, osteoporosis and arthritis, aspirin-sensitive rhinosinusitis, cystic fibrosis, multiple sclerosis, kidney diseases and cancer pain (U.S. Patent No. 6,107,305).

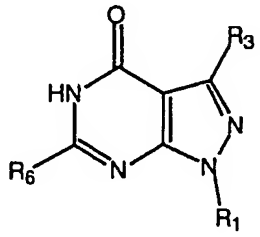
It has also been discovered that some cyclin-dependent kinase inhibitors can be used in combination therapy with some other anticancer agents. For example, the cytotoxic activity of the cyclin-dependent kinase inhibitor, flavopiridol, has been used with other anticancer agents in cancer combination therapy. Cancer Research, 57, 3375 (1997).

Also, it has recently been disclosed that cdk inhibitors may be useful in the chemoprevention of cancer. Chemoprevention is defined as inhibiting the development of invasive cancer by either blocking the initiating mutagenic event or by blocking the progression of pre-malignant cells that have already suffered an insult or inhibiting tumor relapse (U.S. Patent No. 6,107,305).

It has recently been discovered that cdk5 is involved in the phosphorylation of tau protein, and therefore cdk inhibitors may be useful in the treatment of Alzheimer's disease (J. Biochem., 117, 741-749, 1995).

This body of evidence has led to an intense search for small molecule inhibitors of the cdk family as an approach to cancer chemotherapy.

Schmidt et al. describe in U.S. Pat. No. 3,211,731, issued Oct. 12, 1965, pyrazolo[3,4-d]pyrimidines of the formula:



where, among several alternatives:

R_1 represents hydrogen, alkyl, cycloalkyl, aralkyl, oxalkyl, hydroxyalkyl, halogenoalkyl, cycloalkylalkyl, heteroaralkyl, mono- or binuclear aryl or heteroaryl;

R_3 represents hydrogen or lower alkyl;

R_6 represents substituted or unsubstituted aralkyl or heteroaralkyl.

These compounds are claimed to have utility as coronary dilating agents. Schmidt et al. discloses pyrazolo[3,4-d]pyrimidines as intermediates, in U.S. Pat. No. 3,211,732, issued Oct. 12, 1965.

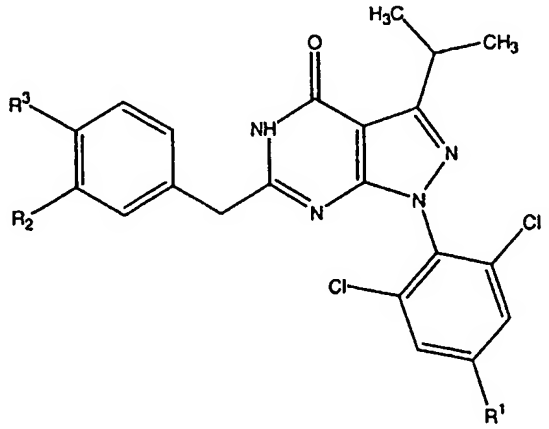
SUMMARY OF THE INVENTION

The present invention is directed to 6-substituted pyrazolo [3,4-d] pyrimidin-4-ones or pharmaceutically acceptable salt or prodrug forms thereof, that are inhibitors of the class of enzymes known as cyclin dependent kinases.

The present invention is also directed to methods of treating cancer or other proliferative diseases by administering a therapeutically effective amount of at least one of the compounds of the present invention or a pharmaceutically acceptable salt or prodrug form thereof to a patient in need of such treatment.

Additionally the present invention is directed to methods of treating cancer or other proliferative diseases, which comprises administering a therapeutically effective combination of at least one of the compounds of the present invention and at least one other known anti-cancer or anti-proliferative agent.

A compound having the formula:



and wherein;

R^1 is selected from the group consisting of -Cl, -NHCHO and $-SO_2NH_2$;

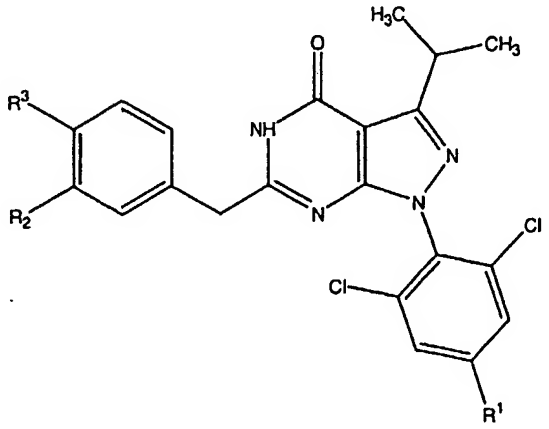
R^2 is selected from the group consisting of -H, -OH, and $-OC_{1-4}$ alkyl;

R^3 is selected from the group consisting of $-NHCOO(CH_2)_3N(CH_3)_2$, -NHCO(4-methyl piperazinyl), $-NHSO_2(CH_2)_2N(CH_3)_2$, -OH, $-OC_{1-4}$ alkyl, -COOH and $-NHCONH(CH_2)_3CH_3$.

As described herein, the inhibitors of this invention are capable of inhibiting the cell-cycle machinery and consequently would be useful in modulating cell-cycle progression, which would ultimately control cell growth and differentiation. Such compounds would be useful for treating subjects having disorders associated with excessive cell proliferation, such as cancer, psoriasis, immunological disorders involving unwanted leukocyte proliferation, in the treatment of restenosis and other smooth muscle cell disorders, and the like.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a class of compounds of formula (I) or it's isomers, prodrugs, tautomers, pharmaceutically acceptable salts, n-oxide forms or sterioisomers:



(I)

and wherein;

R^1 is selected from the group consisting of -Cl, -NHCHO and $-SO_2NH_2$;

R^2 is selected from the group consisting of -H, -OH, and $-OC_{1-4}$ alkyl;

R^3 is selected from the group consisting of $-NHCOO(CH_2)_3N(CH_3)_2$, -NHCO(4-methyl piperazinyl), $-NHSO_2(CH_2)_2N(CH_3)_2$, -OH, $-OC_{1-4}$ alkyl, -COOH and $-NHCONH(CH_2)_3CH_3$.

In one embodiment of the present invention, the compound of formula (I) is selected from:

a) 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-(3-(N, N-dimethylamino)prop-1-yloxycarbonylamino)benzyl) pyrazolo[3,4-d]pyrimidin-4-one;

b) 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-(4-methylpiperazin-1-ylcarbonylamino)benzyl)pyrazolo[3,4-d]pyrimidin-4-one;

c) 1-(2,4,6-Trichlorophenyl)-3-isopropyl-6-(4-(2-(dimethylamino)ethanesulfonamido)benzyl)pyrazolo[3,4-d]pyrimidin-4-one;

d) N-{3,5-Dichloro-4-[3-isopropyl-6-(3-methoxy-benzyl)-4-oxo-4,5-dihydro-pyrazolo[3,4-d]pyrimidin-1-yl]-phenyl}-formamide;

e) 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-methoxybenzyl)pyrazolo[3,4-d]pyrimidin-4-one;

f) 4-{6-[4-(3-Butyl-ureido)-benzyl]-3-isopropyl-4-oxo-4,5-dihydro-pyrazolo[3,4-d]pyrimidin-1-yl}-3,5-dichloro-benzenesulfonamide;

g) 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-carboxybenzyl)pyrazolo[3,4-d]pyrimidin-4-one.

As used above, and throughout the description of the invention, the following terms, unless otherwise indicated, shall be understood to have the following meaning.

The term "compounds of the invention", and equivalent expressions, are meant to embrace compounds of formula (I), and includes prodrugs, pharmaceutically acceptable salts, and solvates, e.g. hydrates. Similarly, reference to intermediates, whether or not they themselves are claimed, is meant to embrace their salts, and solvates, where the context so permits.

The term "derivative" means a chemically modified compound wherein the modification is considered routine by the ordinary skilled chemist, such as an ester or an amide of an acid, protecting groups, such as a benzyl group for an alcohol or thiol, and tert-butoxycarbonyl group for an amine.

The term "analogue" means a compound which comprises a chemically modified form of a specific compound or class thereof, and which maintains the pharmaceutical and/or pharmacological activities characteristic of said compound or class.

The term "solvate" means a physical association of a compound of this invention with one or more solvent molecules. This physical association includes hydrogen bonding. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. "Solvate" encompasses both solution-phase and isolable solvates. Exemplary solvates include hydrates, ethanolates, methanolates, and the like.

The term "effective amount" means an amount of a compound/composition according to the present invention effective in producing the desired therapeutic effect. The term "patient" includes both human and other mammals. The term "pharmaceutical composition" means a composition comprising a compound of formula (I) in combination with at least one additional pharmaceutical adjuvant, excipient, vehicle and/or carrier component pharmaceutically acceptable, such as diluents, preserving agents, fillers, flow regulating agents, disintegrating agents, wetting agents, emulsifying agents, suspending agents, sweetening agents, flavoring agents, perfuming agents, antibacterial agents, antifungal agents, lubricating agents and dispensing agents, depending on the nature of the mode of administration and dosage forms. Any ingredient listed in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Company, may be used.

The term "alkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms. Examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, and t-butyl.

The term "alkoxy" is intended to represent an alkyl group with the indicated number of carbon atoms attached to an oxygen atom. Examples of alkoxy include, but are not limited to, methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, s-butoxy, and t-butoxy.

As used herein, the term "heterocycle" or "heterocyclic system" means a cyclic compound which consists of carbon atoms and from 1 to 2 heteroatoms independently selected from the group consisting of nitrogen and oxygen atoms. The nitrogen atom may optionally be oxidized. The heterocyclic ring may be attached to its pendant group at any heteroatom or carbon atom which results in a stable structure. The

heterocyclic rings described herein may be substituted on carbon or on a nitrogen atom if the resulting compound is stable. If specifically noted, a nitrogen in the heterocycle may optionally be quaternized.

Examples of heterocycles include, but are not limited to piperidinyl, morpholinyl, or piperazinyl groups.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or

acetonitrile are preferred. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Company, Easton, PA, 1990, p. 1445, the disclosure of which is hereby incorporated by reference.

The compounds of the present invention are useful in the form of the free base or acid or in the form of a pharmaceutically acceptable salt thereof. All forms are within the scope of the invention.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication commensurate with a reasonable risk/benefit ratio.

The term "pharmaceutically acceptable prodrugs" as used herein means those prodrugs of the compounds useful according to the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable risk/benefit ratio, and effective for their intended use, as well as zwitterionic forms, where possible, of the compounds of the invention.

The term "prodrugs", as the term is used herein, are intended to include any covalently bonded carriers which release an active parent drug of the present invention in vivo when such prodrug is administered to a mammalian subject. Since prodrugs are known to enhance numerous desirable qualities of pharmaceuticals (i.e., solubility, bioavailability, manufacturing, etc.) the compounds of the present invention may be delivered in prodrug form. Thus, the skilled artisan will appreciate that the present mention encompasses prodrugs of the presently claimed compounds, methods of delivering the same, and

compositions containing the same. Prodrugs of the present invention are prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either in routine manipulation or in vivo, to the parent compound. The transformation in vivo may be, for example, as the result of some metabolic process, such as chemical or enzymatic hydrolysis of a carboxylic, phosphoric or sulphate ester, or reduction or oxidation of a susceptible functionality. Prodrugs include compounds of the present invention wherein a hydroxy, amino, or sulfhydryl group is bonded to any group that, when the prodrug of the present invention is administered to a mammalian subject, it cleaves to form a free hydroxyl, free amino, or free sulfydryl group, respectively. Functional groups which may be rapidly transformed, by metabolic cleavage, in vivo form a class of groups reactive with the carboxyl group of the compounds of this invention. They include, but are not limited to such groups as alkanoyl (such as acetyl, propionyl, butyryl, and the like), unsubstituted and substituted aroyl (such as benzoyl and substituted benzoyl), alkoxycarbonyl (such as ethoxycarbonyl), trialkylsilyl (such as trimethyl- and triethysilyl), monoesters formed with dicarboxylic acids (such as succinyl), and the like. Because of the ease with which the metabolically cleavable groups of the compounds useful according to this invention are cleaved in vivo, the compounds bearing such groups can act as pro-drugs. The compounds bearing the metabolically cleavable groups have the advantage that they may exhibit improved bioavailability as a result of enhanced solubility and/or rate of absorption conferred upon the parent compound by virtue of the presence of the metabolically cleavable group. A thorough discussion of prodrugs is provided in the following: Design of Prodrugs, H. Bundgaard, ed., Elsevier, 1985; Methods in Enzymology, K. Widder et al, Ed., Academic Press, 42, p.309-396, 1985; A Textbook of

Drug Design and Development, Krogsgaard-Larsen and H. Bundgaard, ed., Chapter 5; "Design and Applications of Prodrugs" p.113-191, 1991; Advanced Drug Delivery Reviews, H. Bundgard, 8, p.1-38, 1992; Journal of Pharmaceutical Sciences, 77, p. 285, 1988; Chem. Pharm. Bull., N. Nakeya et al, 32, p. 692, 1984; Pro-drugs as Novel Delivery Systems, T. Higuchi and V. Stella, Vol. 14 of the A.C.S. Symposium Series, and Bioreversible Carriers in Drug Design, Edward B. Roche, ed., American Pharmaceutical Association and Pergamon Press, 1987, each of which is herein incorporated by reference in their entirety as though set forth in full.

The term "treating" refers to: (i) preventing a disease, disorder or condition from occurring in an animal which may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it; (ii) inhibiting the disease, disorder or condition, i.e., arresting its development; and (iii) relieving the disease, disorder or condition, i.e., causing regression of the disease, disorder and/or condition.

Preparation of Compounds of the Invention

It will be apparent to those skilled in the art that certain compounds of formula (I) can exhibit isomerism, for example geometrical isomerism, e.g., E or Z isomerism, and optical isomerism, e.g., R or S configurations. Geometrical isomers include the cis and trans forms of compounds of the invention having alkenyl moieties. It is well known in the art how to prepare optically active forms, such as by resolution of racemic forms or by synthesis from optically active starting materials. All chiral, diastereomeric, racemic forms and all geometric isomeric forms of a structure are intended, unless the specific stereochemistry or isomer form is specifically indicated.

Such isomers can be separated from their mixtures, by the application or adaptation of known methods, for example chromatographic techniques and recrystallization techniques, or they are separately prepared from the appropriate isomers of their intermediates, for example by the application or adaptation of methods described herein.

Where the compound of the present invention is substituted with a basic moiety, acid addition salts are formed and are simply a more convenient form for use; and in practice, use of the salt form inherently amounts to use of the free base form. The acids which can be used to prepare the acid addition salts include preferably those which produce, when combined with the free base, pharmaceutically acceptable salts, that is, salts whose anions are non-toxic to the patient in pharmaceutical doses of the salts, so that the beneficial inhibitory effects on CDK inherent in the free base are not vitiated by side effects ascribable to the anions. Although pharmaceutically acceptable salts of said basic compounds are preferred, all acid addition salts are useful as sources of the free base form even if the particular salt, per se, is desired only as an intermediate product as, for example, when the salt is formed only for purposes of purification, and identification, or when it is used as an intermediate in preparing a pharmaceutically acceptable salt by ion exchange procedures.

According to a further feature of the invention, acid addition salts of the compounds of this invention are prepared by reaction of the free base with the appropriate acid, by the application or adaptation of known methods. For example, the acid addition salts of the compounds of this invention are prepared either by dissolving the free base in aqueous or aqueous-alcohol solution or other suitable solvents containing the appropriate acid and isolating the salt by evaporating

the solution, or by reacting the free base and acid in an organic solvent, in which case the salt separates directly or can be obtained by concentration of the solution.

The acid addition salts of the compounds of this invention can be regenerated from the salts by the application or adaptation of known methods. For example, parent compounds of the invention can be regenerated from their acid addition salts by treatment with an alkali, e.g. aqueous sodium bicarbonate solution or aqueous ammonia solution.

Where the compound of the invention is substituted with an acidic moiety, base addition salts may be formed and can be simply a more convenient form for use; and in practice, use of the salt form can inherently amounts to use of the free acid form. The bases which can be used to prepare the base addition salts include those which produce, when combined with the free acid, pharmaceutically acceptable salts, that is, salts whose cations are non-toxic to the animal organism in pharmaceutical doses of the salts, so that the beneficial inhibitory effects on CDK inherent in the free acid are not vitiated by side effects ascribable to the cations. Pharmaceutically acceptable salts, including for example alkali and alkaline earth metal salts, within the scope of the invention are those derived from the following bases: sodium hydride, sodium hydroxide, potassium hydroxide, calcium hydroxide, aluminum hydroxide, lithium hydroxide, magnesium hydroxide, zinc hydroxide, ammonia, ethylenediamine, N-methyl-glucamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylenediamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethylamine, diethylamine, piperazine, tris(hydroxymethyl)-aminomethane, tetramethylammonium hydroxide, and the like.

Metal salts of compounds of the present invention may be obtained by contacting a hydride, hydroxide,

carbonate or similar reactive compound of the chosen metal in an aqueous or organic solvent with the free acid form of the compound. The aqueous solvent employed may be water or it may be a mixture of water with an organic solvent, preferably an alcohol such as methanol or ethanol, a ketone such as acetone, an aliphatic ether such as tetrahydrofuran, or an ester such as ethyl acetate. Such reactions are normally conducted at ambient temperature but they may, if desired, be conducted with heating.

Amine salts of compounds of the present invention may be obtained by contacting an amine in an aqueous or organic solvent with the free acid form of the compound. Suitable aqueous solvents include water and mixtures of water with alcohols such as methanol or ethanol, ethers such as tetrahydrofuran, nitriles such as acetonitrile, or ketones such as acetone. Amino acid salts may be similarly prepared.

The base addition salts of the compounds of this invention can be regenerated from the salts by the application or adaptation of known methods. For example, parent compounds of the invention can be regenerated from their base addition salts by treatment with an acid, e.g. hydrochloric acid.

Pharmaceutically acceptable salts also include quaternary lower alkyl ammonium salts. The quaternary salts are prepared by the exhaustive alkylation of basic nitrogen atoms in compounds, including nonaromatic and aromatic basic nitrogen atoms, according to the invention, i.e., alkylating the non-bonded pair of electrons of the nitrogen moieties with an alkylating agent such as methylhalide, particularly methyl iodide, or dimethyl sulfate. Quaternarization results in the nitrogen moiety becoming positively charged and having a negative counter ion associated therewith.

As will be self-evident to those skilled in the art, some of the compounds of this invention do not form

stable salts. However, acid addition salts are more likely to be formed by compounds of this invention having a nitrogen-containing heteroaryl group and/or wherein the compounds contain an amino group as a substituent. Preferable acid addition salts of the compounds of the invention are those wherein there is not an acid labile group.

As well as being useful in themselves as active compounds, salts of compounds of the invention are useful for the purposes of purification of the compounds, for example by exploitation of the solubility differences between the salts and the parent compounds, side products and/or starting materials, by techniques well known to those skilled in the art.

Compounds according to the invention, for example, starting materials, intermediates or products, are prepared as described herein or by the application or adaptation of known methods, by which is meant methods used heretofore or described in the literature, for example those described by R. C. Larock in Comprehensive Organic Transformations, VCH publishers, 1989.

In the reactions described hereinafter it may be necessary to protect reactive functional groups, for example hydroxy, amino, imino, thio or carboxy groups, where these are desired in the final product, to avoid their unwanted participation in the reactions. Conventional protecting groups may be used in accordance with standard practice, for examples see T.W. Green and P.G.M.Wuts in "Protective Groups in Organic Chemistry" John Wiley and Sons, 1991; J. F. W. McOmie in "Protective Groups in Organic Chemistry" Plenum Press, 1973.

The compounds useful according to the invention optionally are supplied as salts. Those salts which are pharmaceutically acceptable are of particular interest since they are useful in administering the foregoing compounds for medical purposes. Salts which are not pharmaceutically acceptable are useful in manufacturing

processes, for isolation and purification purposes, and in some instances, for use in separating stereoisomeric forms of the compounds of this invention. The latter is particularly true of amine salts prepared from optically active amines.

Where the compound useful according to the invention contains a carboxy group, or a sufficiently acidic bioisostere, base addition salts may be formed and are simply a more convenient form for use; and in practice, use of the salt form inherently amounts to use of the free acid form.

Also, where the compound useful according to the invention contains a basic group, or a sufficiently basic bioisostere, acid addition salts may be formed and are simply a more convenient form for use; and in practice, use of the salt form inherently amounts to use of the free base form.

The foregoing compounds useful according to the invention may also be combined with another therapeutic compound to form pharmaceutical compositions (with or without diluent or carrier) which, when administered, provide simultaneous administration of two or more active ingredients resulting in the combination therapy of the invention.

While it is possible for compounds useful according to the invention to be administered alone it is preferably to present them as pharmaceutical compositions. The pharmaceutical compositions, both for veterinary and for human use, useful according to the present invention comprise at lease one compound of the invention, as above defined, together with one or more acceptable carriers therefor and optionally other therapeutic ingredients. The skilled artisan will appreciate the abundance of publications setting forth the state of the art for pharmaceutical administration.

Examples of suspending agents include ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and

sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, for example sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monosterate and gelatin. Examples of suitable carriers, diluents, solvents or vehicles include water, ethanol, polyols, suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Examples of excipients include lactose, milk sugar, sodium citrate, calcium carbonate, dicalcium phosphate phosphate. Examples of disintegrating agents include starch, alginic acids and certain complex silicates. Examples of lubricants include magnesium stearate, sodium lauryl sulphate, talc, as well as high molecular weight polyethylene glycols.

In certain preferred embodiments, active ingredients necessary in combination therapy may be combined in a single pharmaceutical composition for simultaneous administration.

The choice of vehicle and the content of active substance in the vehicle are generally determined in accordance with the solubility and chemical properties of the active compound, the particular mode of administration and the provisions to be observed in pharmaceutical practice. For example, excipients such as lactose, sodium citrate, calcium carbonate, dicalcium phosphate and disintegrating agents such as starch, alginic acids and certain complex silicates combined with lubricants such as magnesium stearate, sodium lauryl sulphate and talc may be used for preparing tablets. To

prepare a capsule, it is advantageous to use lactose and high molecular weight polyethylene glycols. When aqueous suspensions are used they can contain emulsifying agents or agents which facilitate suspension. Diluents such as sucrose, ethanol, polyethylene glycol, propylene glycol, glycerol and chloroform or mixtures thereof may also be used.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the oily phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the emulsifying wax, and the way together with the oil and fat make up the emulsifying ointment base which forms the oily dispersed phase of a cream formulation. Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween® 60, Span® 80, cetostearyl alcohol, benzyl alcohol, myristyl alcohol, glyceryl mono-stearate and sodium lauryl sulfate.

If desired, the aqueous phase of the cream base may include, for example, a least 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane 1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol (including PEG 400) and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active ingredient through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethyl sulphoxide and related analogues.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties. Thus the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used. Solid compositions may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols, and the like.

The pharmaceutical compositions can be administered in a suitable formulation to humans and animals by topical or systemic administration, including oral, inhalational, rectal, nasal, buccal, sublingual, vaginal, parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural), intracisternal and intraperitoneal. It will be appreciated that the preferred route may vary with for example the condition of the recipient.

The formulations can be prepared in unit dosage form by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tables may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compounds moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

Solid compositions for rectal administration include suppositories formulated in accordance with known methods and containing at least one compound of the invention.

If desired, and for more effective distribution, the compounds can be microencapsulated in, or attached to, a slow release or targeted delivery systems such as a biocompatible, biodegradable polymer matrices (e.g. poly(d,l-lactide co-glycolide)), liposomes, and microspheres and subcutaneously or intramuscularly injected by a technique called subcutaneous or intramuscular depot to provide continuous slow release of the compound(s) for a period of 2 weeks or longer. The compounds may be sterilized, for example, by filtration through a bacteria retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use.

Actual dosage levels of active ingredient in the compositions of the invention may be varied so as to obtain an amount of active ingredient that is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore depends upon the desired

therapeutic effect, on the route of administration, on the desired duration of treatment and other factors.

Total daily dose of the compounds useful according to this invention administered to a host in single or divided doses may be in amounts, for example, of from about 0.0001 to about 100 mg/kg body weight daily and preferably 0.01 to 10 mg/kg/day. Dosage unit compositions may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the patient's body weight, general health, sex, diet, time and route of administration, rates of absorption and excretion, combination with other drugs and the severity of the particular disease being treated.

The amount of each component administered is determined by the attending clinicians taking into consideration the etiology and severity of the disease, the patient's condition and age, the potency of each component and other factors.

The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials with elastomeric stoppers, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Administration of a compound of the present invention in combination with additional therapeutic agents, may afford an efficacy advantage over the compounds and agents alone, and may do so while permitting the use of lower doses of each. A lower dosage minimizes the potential of side effects, thereby providing an increased margin of safety. The combination

of a compound of the present invention with such additional therapeutic agents is preferably a synergistic combination. Synergy, as described for example by Chou and Talalay, Adv. Enzyme Regul. 22:27-55 (1984), occurs when the therapeutic effect of the compound and agent when administered in combination is greater than the additive effect of either the compound or agent when administered alone. In general, a synergistic effect is most clearly demonstrated at levels that are (therapeutically) sub-optimal for either the compound of the present invention or a known anti-proliferative agent alone, but which are highly efficacious in combination. Synergy can be in terms of improved inhibitory response without substantial increases in toxicity over individual treatments alone, or some other beneficial effect of the combination compared with the individual components.

Procedures for evaluating the biological activity of compounds or compositions according to the invention are carried out as described herein or by the application or adaptation of known procedures, by which is meant procedures used heretofore or as described in the literature. United States patent application Serial Number 09/794,825, filed February 27, 2001, by Markwalder etal. Is herein incorporated by reference in it's entirity as though set forth in full. The compounds of the present invention, their methods or preparation and their biological activity will appear more clearly from the examination of the following examples which are presented as an illustration only and are not to be considered as limiting the invention in its scope. The following examples are but preferred methods of synthesizing the compounds of the invention, which may be prepared according to any method known to the organic chemist of ordinary skill. Other features of the invention will become apparent during the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be

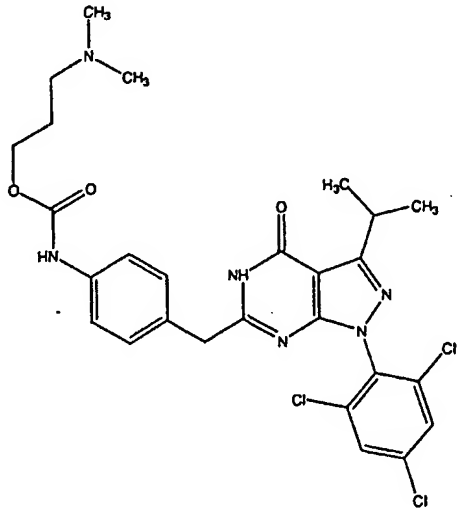
limiting thereof. Each of the patents, patent applications, and other cited references, are hereby incorporated herein by reference in their entirity as though set forth in full.

EXAMPLES

The following abbreviations are used throughout the following Examples: "°C" for degrees Celsius, "CIMS" for chemical ionization mass spectroscopy, "eq" for equivalent or equivalents, "g" for gram or grams, "h" for hour or hours, "mg" for milligram or milligrams, "mL" for milliliter or milliliters, "mmol" for millimolar, "M" for molar, "min" for minute or minutes, "p-TsOH" for para-toluenesulphonic acid, "DMF" for dimethylformamide, and "TFA" for trifluoroacetic acid.

EXAMPLE 1

Synthesis of 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-(3-(N, N-dimethylamino)prop-1-yloxycarbonylamino)benzyl) pyrazolo[3,4-d]pyrimidin-4-one

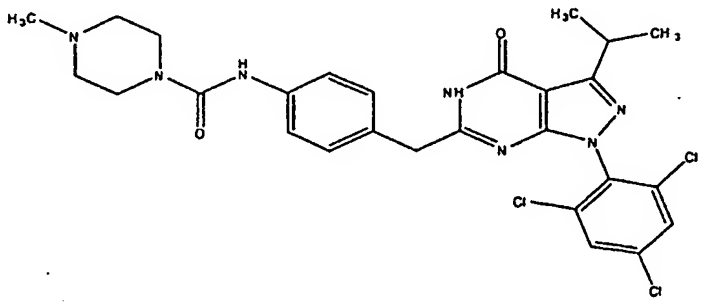


To a stirred, cooled(-78°C) solution of 0.13 mL(1.0 mmol) of 3-(N, N-dimethylamino)propan-1-ol in 1 mL of THF is added 0.28 mL(0.70 mmol) of a 2.5 M solution of n-BuLi

in hexane over 2 minutes. The solution is stirred for 5 minutes at -78°C, treated with 49 mg(0.10 mmol) of the 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-isocyanatobenzyl)pyrazolo[3,4-d]pyrimidin-4-one (prepared in Example 106 in co-pending US patent application serial no. 09/794,825, filed February 27, 2001), and warmed to 0°C over 10 minutes. The mixture is treated with 2 mL of 10% aq. HOAc, treated with saturated aqueous $NaHCO_3$ until bubbling ceased, and extracted with EtOAc. The organic extract is washed (brine), dried ($MgSO_4$), diluted with hexane, and partially concentrated under reduced pressure. Filtration afforded 39 mg (66%) of 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-(3-(N, N-dimethylamino)prop-1-yloxycarbonylamino)benzyl)pyrazolo[3,4-d]pyrimidin-4-one as an off-white solid, mp 200-202°C. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.43(br. s, 1H); 9.54(br. s, 1H); 7.97(s, 2H); 7.32(d, 2H, J = 8.4 Hz); 7.14(d, 2H, J = 8.8 Hz); 4.03(t, 2H, J = 6.6 Hz); 3.76(s, 2H); 3.21-3.30(m, 1H); 2.24(t, 2H, J = 7.2 Hz); 2.08(s, 6H); 1.64-1.74(m, 2H); 1.28(d, 6H, J = 6.9 Hz).

EXAMPLE 2

Synthesis of 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-(4-methylpiperazin-1-ylcarbonylamino)benzyl)pyrazolo[3,4-d]pyrimidin-4-one.

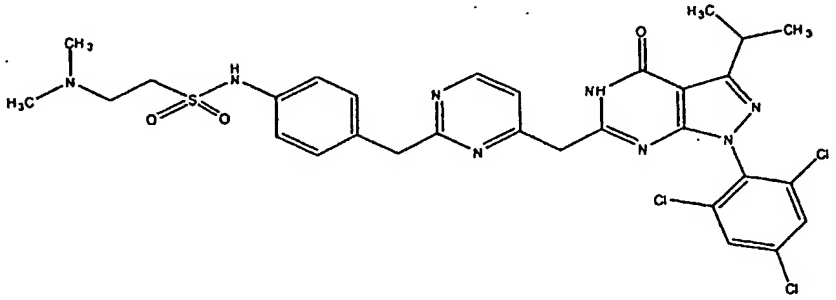


To a warm stirred solution of 489 mg(1.0 mmol) of the isocyanate prepared in example 106 in 20 mL of 1:1

THF-CH_2Cl_2 is added 0.33 mL(3.0 mmol) of 1-methylpiperazine. The warm solution is stirred 5 min. then heated to reflux. Heptane(10-15 mL) is added with continued heating to give a white precipitate. The resulting material is filtered, rinsed with 1:1 ether-hexanes, and briefly air-dried to afford 483 mg(82%) of 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-(4-methylpiperazin-1-ylcarbonylamino)benzyl)pyrazolo[3,4-d]pyrimidin-4-one as a white solid, mp 251-253°C. ^{1}H NMR (300 MHz, DMSO-d6) d 12.41(br. s, 1H); 8.44(s, 1H); 7.97(s, 2H); 7.32(d, 2H, J = 8.8 Hz); 7.10(d, 2H, J = 8.8 Hz); 3.75(s, 2H); 3.37(t, 4H, J = 4.9 Hz); 3.19-3.28(m, 1H); 2.25(t, 4H, J = 4.9 Hz); 2.15(s, 3H); 1.28(d, 6H, J = 6.9 Hz).

EXAMPLE 3

Synthesis of 1-(2,4,6-Trichlorophenyl)-3-isopropyl-6-(4-(2-(dimethylamino)ethanesulfonamido)benzyl)pyrazolo[3,4-d]pyrimidin-4-one

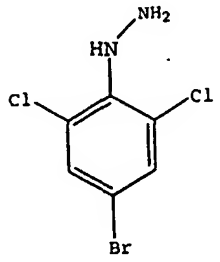


To a stirred, solution of 23 mg(0.042 mmol) of 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-(ethenesulfonamido)benzyl)pyrazolo[3,4-d]pyrimidin-4-one in 1 mL of THF was added 1 mL of 2M dimethylamine in THF. The solution was stirred 3 h and concentrated under reduced pressure. The product was dissolved in 1 mL of benzene and 0.05 mL of MeOH, frozen, and lyophilized to afford 25 mg (100%) of 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-(2-(dimethylamino)ethanesulfonamido) benzyl)pyrazolo[3,4-

d]pyrimidin-4-one as an amorphous white solid. ^{1}H NMR(300 MHz, DMSO) δ 7.96(s, 2H); 7.19(d, 2H, J = 8.5 Hz); 7.08(d, 2H, J = 8.8 Hz); 3.79(s, 2H); 3.18-3.32(m, 1H); 3.13(t, 2H, J = 7.5 Hz); 2.53(t, 2H, J = 7.5Hz); 1.99(s, 6H); 1.28(d, 6H, J = 7.0 Hz).

EXAMPLE 4

Preparation of Intermediate 1

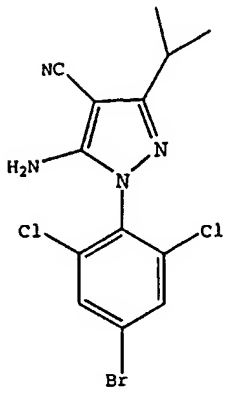


1

To an ice-cooled suspension of 4-bromo-2,6-dichloroaniline (24.1 g, 100 mmol) in concentrated hydrochloric acid (100 mL) is added a solution of sodium nitrite (6.9 g, 100 mmol) in water (100 mL) dropwise via an addition funnel. After stirring at 0° C for 1 hour, a solution of tin chloride (67.7 g, 300 mmol) in concentrated hydrochloric acid (120 mL) is added dropwise via an addition funnel. The reaction is stirred vigorously then capped and put in the refrigerator for 2 days. The solid is filtered, washed with brine and dried. It is then transferred to a beaker containing 2N NaOH solution (500 mL) and stirred for several hours. The solid is filtered and air dried. Compound 1 is obtained as a tan solid (23.67 g, 92%): ^{1}H NMR (DMSO-d6) δ 7.54 (s, 2 H), 6.02 (br, 1 H), 4.34 (m, 2 H); ESI m/z = 484.2 (M-NH2)+.

EXAMPLE 5

Preparation of Intermediate 2



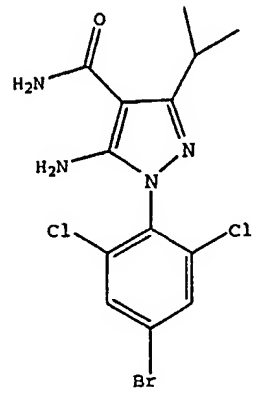
2

A solution of intermediate 1 (10.22 g, 39.93 mmol) and 1,1-dicyano-2-methoxy-3-methyl-1-butene (5g, 33.29 mmol) in methanol (75 mL) is heated at reflux under N_2 for 6 hours.

The solution is cooled to room temperature, diluted with water (75 mL) and extracted with ethyl acetate. The organics are washed with 10% citric acid, water and brine, dried ($MgSO_4$), and evaporated under reduced pressure. Purification by column chromatography on silica gel using 3:1 hexane/ethyl acetate as eluant afforded compound 2 as a pale yellow solid (6.54 g, 44%); ^1H NMR (DMSO-d6) δ 7.98 (s, 2 H), 6.78 (s, 2 H), 2.84 (septet, 1 H), 1.18 (d, 6 H); ESI m/z = 373 (M-H)-.

EXAMPLE 6

Preparation of Intermediate 3

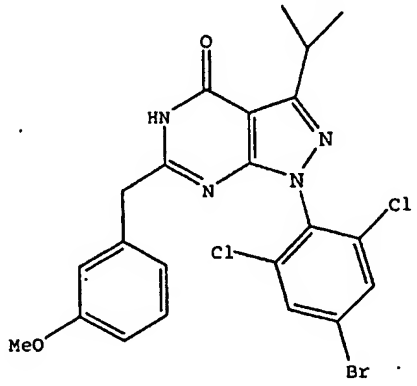


3

A solution of intermediate 2 (6.4 g, 17.11 mmol) in concentrated sulfuric acid (10 mL) is stirred at room temperature overnight. The reaction is carefully quenched by slow addition of ice follow by dilution with water (100 mL). The solid precipitate is filtered, washed with water and then with 3:1 hexane/diethyl ether. Compound 3 is isolated as a pale yellow solid (6.08 g, 91%); ^{1}H NMR (DMSO-d6) δ 7.96 (s, 2 H), 6.61 (s, 2 H), 6.31 (m, 2H), 3.28 (m, 1 H), 1.13 (d, J = 6.5 Hz, 6 H); APCI m/z = 393 (M+H)+.

EXAMPLE 7

Preparation of Intermediate 4

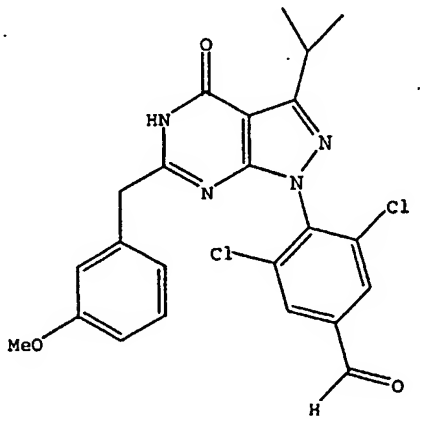


4

To a solution of methyl 3-methoxyphenyl-acetate (8 g, 44.4 mmol) in methanol (40 mL) is added compound 3 (5.8 g, 14.8 mmol) followed by sodium methoxide (10.2 mL of 25% solution in MeOH, 44.4 mmol). After heating at relux overnight, the heat is removed and acetic acid (40 mL of a 10% aqueous solution) is added. Once the reaction reached room temperature saturated sodium bicarbonate solution (40 mL) is added slowly. After stirring for 15 minutes the precipitate is collected by suction filtration, washed sequentially with 1:1 MeOH/H_2O and then 3:1 hexane/diethyl ether and dried. The resulting compound was obtained as a tan solid (6.08 g, 79%); ^1H NMR (DMSO-d6) 8.06 (s, 2 H), 7.16 (t, 1 H), 6.85 - 6.74 (m, 3H), 3.80 (s, 2 H); 3.66 (s, 3H), 3.24 (m, 1 H), 1.28 (d, J = 7.0 Hz, 6 H); ESI m/z = 523 (M+H)+.

EXAMPLE 8

Preparation of Intermediate 5



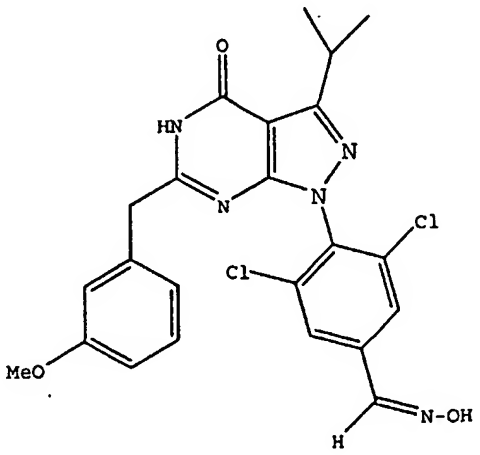
5

A two-necked round bottom flask is flame-dried, charged with compound 4 (1.5 g, 2.87 mmol) and dry tetrahydrofuran (10 mL)and placed under an argon atmosphere. After cooling to 0°C isopropylmagnesium chloride (1.6 mL of 2 M solution in Et_2O, 3.16 mmol) is added via syringe. The reaction is stirred for 5

minutes, cooled to -78°C and sec-butyllithium (2.87 mL of 1.3 M solution in hexane, 3.73 mmol) is added dropwise via syringe. The reaction is stirred at -78°C for ten minutes and dimethylformamide (0.5 mL, 6.31 mmol) was added. Afer stirring for 15 minutes at -78°C and then at 0°C for thirty minutes, the reaction is quenched with CD3OD, diluted with water and extracted with ethyl acetate. The organics are washed with brine, dried ($MgSO_4$) and evaportated. Purification by column chromatography on silica gel using 3:1 hexane/ethyl acetate as eluant afforded compound 5 as a white solid (400 mg, 30%); ^1H NMR (DMSO-d6) 12.50 (s, 1H), 10.01 (s, 1H), 8.18 (s, 2 H), 7.16 (t, 1 H), 6.84 - 6.74 (m, 3H), 3.81 (s, 2 H); 3.66 (s, 3H), 3.26 (m, 1 H), 1.29 (d, J = 7.0 Hz, 6 H); ESI m/z = 469 (M-H)-.

EXAMPLE 9

Preparation of Intermediate 6



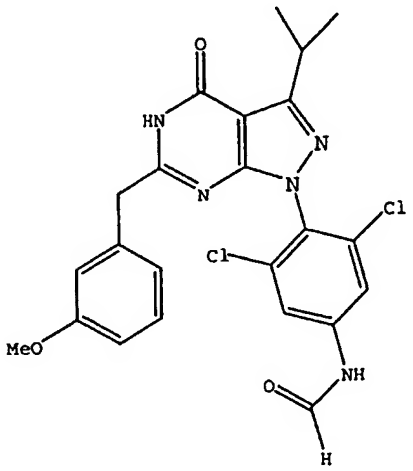
6

To a solution of compound 5 (150 mg, 0.32 mmol) in 5:1 EtOH/H_2O (6 mL) is added hydroxylamine hydrochloride (44 mg, 0.64 mmol). After stirring at room temperature overnight the solvent is removed via rotary evaporation

and water is added. A solid precipitate was collected by suction filtration, washed with water and 3:1 hexane/diethyl ether and dried. The resulting compound is obtained as a white solid (125 mg, 80%); ^{1}H NMR (DMSO-d6) 12.45 (s, 1H), 11.86 (s, 1H), 8.22 (s, 1H), 7.87 (s, 2 H), 7.16 (t, 1 H), 6.85 - 6.74 (m, 3H), 3.81 (s, 2 H); 3.66 (s, 3H), 3.24 (m, 1 H), 1.28 (d, J = 7.0 Hz, 6 H); ESI m/z = 484 (M-H)-.

EXAMPLE 10

Synthesis of N-{3,5-Dichloro-4-[3-isopropyl-6-(3-methoxy-benzyl)-4-oxo-4,5-dihydro-pyrazolo[3,4-d]pyrimidin-1-yl]-phenyl}-formamide

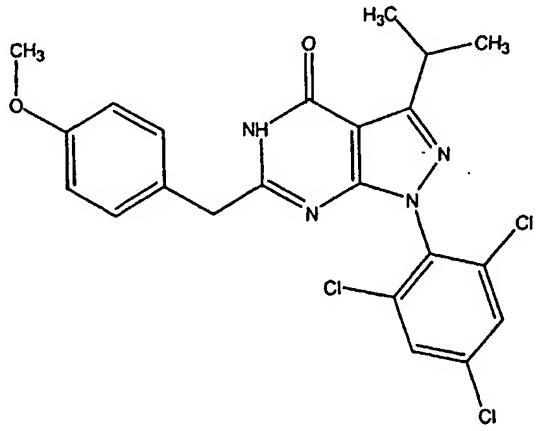


A solution of compound 6 (50 mg, 0.1 mmol) in polyphosphoric acid (1.3 g) is heated at 120°C for 3 hours. After cooling to room temperature ice and then water are added and the aqueous mixture is extracted with ethyl acetate. Organics are washed with water and brine, dried ($MgSO_4$) and evaporated. The product is crystallized under diethyl ether and filtered. The resulting compound is a white solid (36 mg, 74%); ^{1}H NMR (DMSO-d6) 12.47 (s, 1H), 8.29 (s, 1H), 8.10 (s, 2H), 7.82 (s, 1 H), 7.16 (t, 1 H), 6.85 - 6.74 (m, 3H), 3.81 (s, 2 H); 3.66 (s, 3H),

3.24 (m, 1 H), 1.29 (d, J = 7.0 Hz, 6 H); ESI m/z = 486 (M+H)+.

EXAMPLE 11

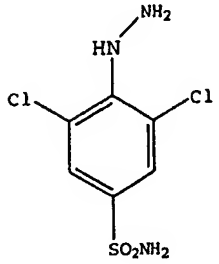
Synthesis of 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-methoxybenzyl)pyrazolo[3,4-d]pyrimidin-4-one



A ten-milliliter screw-cap test tube is oven-dried and charged with 70 mg(0.2 mmol) of 5-amino-3-isopropyl-1-(2,4,6-trichlorophenyl)pyrazole-4-carboxamide (as prepared in example 48B in co-pending US patent application serial no. 09/794,825, filed February 27, 2001), 216 mg(1.2 mmol) of methyl 4-methoxyphenylacetate, and 2 mL of absolute ethanol. The mixture is treated with 0.45 mL (1.2 mmol) of a 2.66 M solution of NaOEt in ethanol, blanketed with dry nitrogen, capped, and placed in a 74oC heating block for 24h. The mixture is then treated with 5 mL of 10% aqueous HOAc, cooled, and filtered. The resulting solid is rinsed with 1:1 MeOH-water, then 1:1 hexanes-ether and briefly air-dried to afford 38 mg (40%) of 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-methoxybenzyl)pyrazolo[3,4-d]pyrimidin-4-one as a cream-colored solid, mp 183-185°C. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.44(br. s, 1H); 7.97(s, 2H); 7.17(d, 2H, J = 8.4 Hz); 6.81(d, 2H, J = 8.7 Hz); 3.76(s, 2H); 3.66(s, 3H); 3.19-3.27(m, 1H); 1.28(d, 6H, J = 7.0 Hz).

EXAMPLE 12

Preparation of Intermediate 7

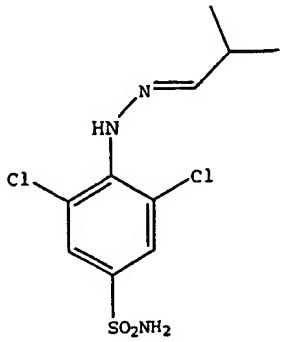


7

To an ice-cooled suspension of 4-amino-3,5-dichlorobenzenesulfonamide (4.83 g, 20 mmol) in concentrated hydrochloric acid (30 mL) is added a solution of sodium nitrite (1.38 g, 20 mmol) in water (10 mL) dropwise via an addition funnel. After stirring at 0° C for 30 min, a solution of tin chloride (13.56 g, 60 mmol) in concentrated hysdrochloric acid (20 mL) is added dropwise via an addition funnel. The reaction is stirred vigorously then capped and put in the refrigerator overnight. The solid is filtered, washed with brine and dried. It is then transferred to a beaker containing 2N NaOH solution (200 mL), stirred for 1 hour, and adjusted to pH = 7 with 10% acetic acid. The solid is filtered and purified by column chromatography on silica gel using 1:1 hexane/ethyl acetate as eluant. Compound 7 is obtained as a tan solid (2.34 g, 46%): ^{1}H NMR (DMSO-d6) δ 7.60 (s, 2 H), 7.36 (br, 2 H), 6.61 (s, 1H), 4.53 (br, 2 H); ESI m/z = 254 (M-H)-.

EXAMPLE 13

Preparation of Intermediate 8

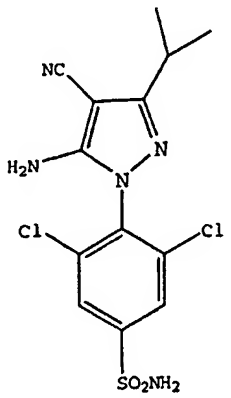


8

A mixture of compound 7 (8.0 g, 31.24 mmol) and isobutyraldehyde (3.12 mL, 34.36 mmol) in ethanol(55 mL) is stirred at room temperature under nitrogen overnight. Water is added and a solid precipitate was collected by suction filtration, washed with water and dried. The resulting compound is isolated as a tan solid (7.92 g, 82%); ^{1}H NMR (DMSO-d6) δ 9.26 (s, 1H), 7.67 (s, 2H), 7.46 (d, J = 3.4 Hz, 1H), 7.40 (s, 2H), 2.44 (m, 1H), 1.01 (d, J = 7.0 Hz, 6H).

EXAMPLE 14

Preparation of Intermediate 9



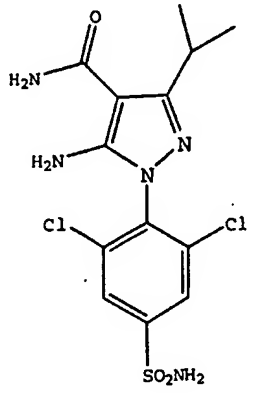
9

To a solution of 8 (7.8 g, 25.14 mmol) in dry N,N-dimethylformamide (40 mL) is added N-bromosuccinimide

(5.37 g, 30.17 mmol) slowly at 0°C. After stirring for one hour at 0°C, the reaction is diluted with water and extracted with diethyl ether. The organics are washed with brine, dried ($MgSO_4$) and evaporated. The anion of malononitrile is prepared by adding sodium methoxide (11.5 mL of 24% solution in MeOH, 50.28 mmol) to a solution of malononitrile (3.32 g, 50.28 mmol) in methanol at 0°C. The bromohydrazone from above is dissolved in methanol (50 mL) and the malononitrile anion solution is added in one portion. The reaction is stirred at room temperature overnight, quenched with water and the pH is adjusted to neutral using 10% acetic acid. The aqueous mixture is extracted with ethyl acetate. The organics are washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure. Purification by column chromatography on silica gel using 1:1 hexane/ethyl acetate as eluant afforded compound 9 as a yellow solid (990 mg, 10%); ^1H NMR (DMSO-d6) δ 7.95 (s, 2 H), 7.79 (s, 2 H), 6.89 (s, 2H), 2.85 (septet, 1 H), 1.19 (d, J = 7.0 Hz, 6 H); ESI m/z = 374 (M+H)+.

EXAMPLE 15

Preparation of Intermediate 10



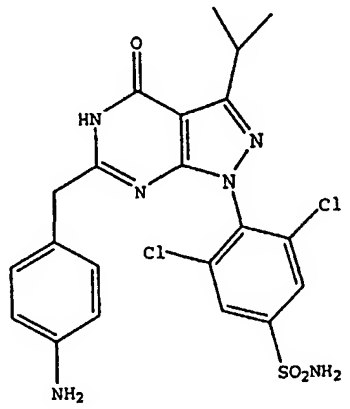
10

A mixture of compound 9 (970 mg, 2.59 mmol) in concentrated sulfuric acid (3 mL) is stirred at room

temperature under nitrogen overnight. The reaction is carefully quenched by slow addition of ice followed by dilution with water. The pH is adjusted to neutral using saturated sodium bicarbonate solution. Filtration and drying will produce compound 10 as a yellow solid (711 mg, 70%); ^{1}H NMR (DMSO-d6) δ 7.94 (s, 2 H), 7.79 (s, 2 H), 6.65 (s, 2H), 6.40 (s, 2H), 3.29 (septet, 1 H), 1.13 (d, J = 6.6 Hz, 6 H); ESI m/z = 390 (M-H)-.

EXAMPLE 16

Preparation of Intermediate 11



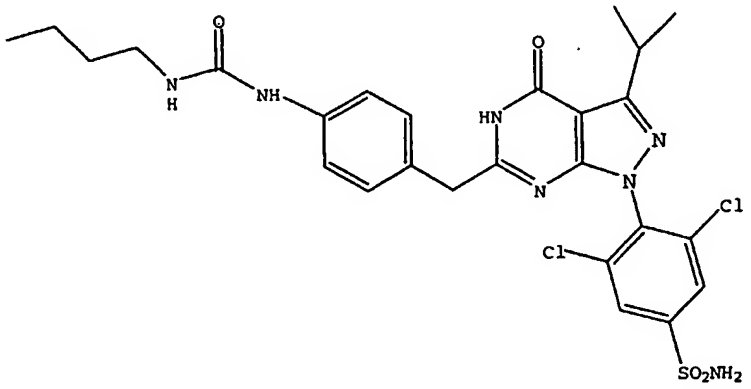
11

To a solution of ethyl 4-aminophenyl-acetate (389 mg, 2.17 mmol) in ethanol (2 mL) is added the compound produced in example 15 (284 mg, 0.724 mmol) followed by sodium ethoxide (0.82 mL of a 2.66M solution in EtOH, 2.17 mmol). After heating at relux overnight, the heat is removed and acetic acid (2 mL of a 10% aqueous solution) is added. Once the reaction reached room temperature saturated sodium bicarbonate solution (2 mL) is added slowly. The aqueous mixture is extracted with ethyl acetate. The organics are washed with brine, dried ($MgSO_4$) and evaporated under reduced pressure. Purification by column chromatography on silica gel using 2:1 ethyl acetate/hexane yields compound 11 as a yellow

solid (81 mg, 22%); ^{1}H NMR (DMSO-d6) 12.37 (s, 1H), 8.06 (s, 2 H), 7.78 (s, 2 H), 6.90 (d, J = 8.3 Hz, 2H), 6.42 (d, J = 8.3 Hz, 2H), 4.94 (br, 2H), 3.63 (s, 2 H); 3.24 (m, 1 H), 1.29 (d, J = 7.0 Hz, 6 H); ESI m/z = 506 (M-H).

EXAMPLE 17

Synthesis of 4-{6-[4-(3-Butyl-ureido)-benzyl]-3-isopropyl-4-oxo-4,5-dihydro-pyrazolo[3,4-d]pyrimidin-1-yl}-3,5-dichloro-benzenesulfonamide



To a solution of compound 11 (25 mg, 0.05 mmol) in anhydrous tetrahydrofuran (0.8 mL) is added n-butylisocyanate (0.009 mL, 0.075 mmol). After stirring at room temperature overnight the reaction is quenched with water. The precipitate is collected by suction filtration, washed with diethyl ether and dried to afford the desired compound as a white solid (22 mg, 72%); ^{1}H NMR (DMSO-d6) 12.48 (s, 1H), 8.30 (s, 1H), 8.06 (s, 2 H), 7.79 (s, 2 H), 7.24 (d, J = 8.4 Hz, 2H), 7.09 (d, J = 8.4 Hz, 2H), 6.04 (t, J = 5.7 Hz, 1H), 3.74 (s, 2 H); 3.56 (t, J = 6.3 Hz, 2H), 3.25 (m, 1 H), 3.00 (q, J = 5.7 Hz, 2H), 1.72 (pent, 2H), 1.29 (d, J = 7.0 Hz, 6 H), 0.84 (t, J = 7.3 Hz; 3 H); ESI m/z = 604 (M-H)-.

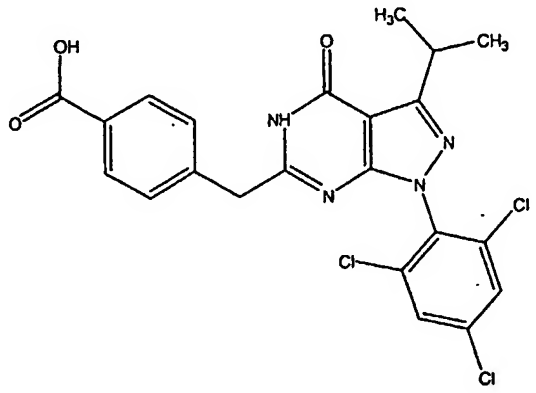
EXAMPLE 18

Preparation of 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-carbethoxybenzyl)pyrazolo[3,4-d]pyrimidin-4-one

To a stirred solution of 174 mg(0.5 mmol) of 5-amino-3-isopropyl-1-(2,4,6-trichlorophenyl)pyrazole-4-carboxamide (as prepared in example 48B in co-pending US patent application serial no. 09/794,825, filed February 27, 2001) and 473 mg(2.0 mmol) of ethyl 4-carbethoxyphenylacetate in 6 mL of absolute ethanol is added 0.94 mL(2.5 mmol) of a 2.66 M solution of sodium ethoxide in ethanol. The solution is stirred 15 h at reflux, cooled slightly, and treated with 8 mL of 10% aq. HOAc. The resulting mixture is then treated with 2 mL of saturated aqueous. $NaHCO_3$, cooled, filtered, and rinsed with 6 mL of 1:1 MeOH-water then 6 mL of 1:1 ether-hexane to produce a solid. The solid is briefly air-dried, affording 323 mg(89%) of 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-isocyanatobenzyl)pyrazolo[3,4-d]pyrimidin-4-one as a white solid, mp 233-235oC. ^1H NMR (300 MHz, DMSO-d6) δ 12.57(br. s, 1H); 7.96(s, 2H); 7.84(d, 2H, J = 8.4 Hz); 7.38(d, 2H, J = 8.4 Hz); 4.25(q, 2H, J = 7.1 Hz); 3.95(s, 2H); 3.21-3.30(m, 1H); 1.23-1.32(m, 9H).

EXAMPLE 19

Synthesis of 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-carboxybenzyl)pyrazolo[3,4-d]pyrimidin-4-one



To a stirred solution of 130 mg(0.25 mmol) of 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-carbethoxybenzyl)pyrazolo[3,4-d]pyrimidin-4-one in 2 mL of THF was added a solution of 42 mg(1 mmol) of lithium hydroxide in 2 mL of water. The mixture is treated with 0.26 mL of methanol to give a homogeneous solution, refluxed for 10 min., and allowed to cool to RT and stir for 3.5 h. The solution is diluted with ether and washed twice with 0.1 N NaOH. The combined aqueous washings are acidified with concentrated aqueous HCl and extracted once with $CHCl_3$ and once with EtOAc. The combined organic extracts are dried($MgSO_4$) and concentrated under reduced pressure to afford 123 mg(100%) of 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-carboxybenzyl)pyrazolo[3,4-d]pyrimidin-4-one as a white solid, mp 294-295. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.56(br. s, 1H); 7.98(s, 2H); 7.82(d, 2H, J = 8.1 Hz); 7.35(d, 2H, J = 8.0 Hz); 3.98(s, 2H); 3.20-3.29(m, 1H); 1.29(d, 6H, J = 6.9 Hz).

UTILITY

Inhibition of Kinase/Cyclin Complex Enzymatic Activity

Several of the compounds disclosed in this invention were assayed for their inhibitory activity against cdk4/D1 and cdk2/E kinase complexes. The in vitro assays employ cell lysates from insect cells expressing either of the kinases and subsequently their corresponding regulatory units. The cdk2/cyclin E is purified from insect cells expressing His-tagged cdk2 and cyclin E. The cdk/cyclin lysate is combined in a microtitre-type plate along with a kinase compatible buffer, ^{32}P-labeled ATP at a concentration of 50 mM, a GST-Rb fusion protein and the test compound at varying concentrations. The kinase reaction is allowed to proceeded with the radiolabled

ATP, then effectively stopped by the addition of a large excess of EDTA and unlabeled ATP. The GST-Rb labeled protein is sequestered on a GSH-Sepharose bead suspension, washed, resuspended in scintillant, and the ^{32}P activity detected in a scintillation counter. The compound concentration which inhibits 50% of the kinase activity was calculated for each compound. A compound was considered active if its IC_{50} was found to be less than 1 μM.

Inhibition of HCT 116 Cancer Cell Proliferation

To test the cellular activity of several compounds disclosed in this invention, we examined the effect of these compounds on cultured HCT116 cells and determined their effect on cell-cycle progression by the colorimetric cytotoxcity test using sulforhodamine B (Skehan et al. J. Natl. Cancer Inst. 82:1107-12, 1990). Briefly, HCT116 cells are cultured in the presence of test compounds at increasing concentrations. At selected time points, groups of cells are fixed with trichloroacetic acid and stained with sulforhodamine B (SRB). Unbound dye was removed by washing and protein-bound dye was extracted for determination of optical density. A compound was considered active if its IC_{50} was found to be less than 10 μM.

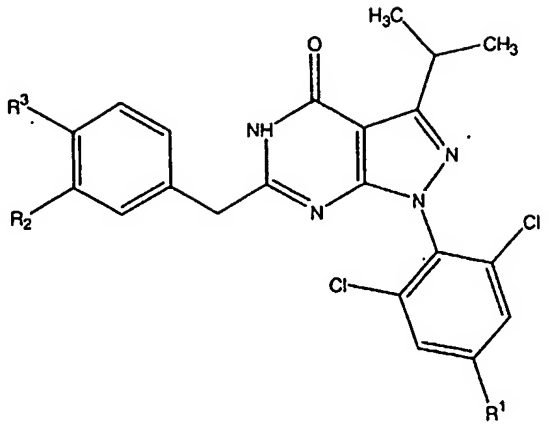
All patents, patent applications and other publications are herein incorporated by reference in their entirity as though set forth in full.

Other features of the invention will become apparent during the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

CLAIMS

I/We Claim:

1. A compound having the formula:



and wherein;

R^1 is selected from the group consisting of -Cl, -NHCHO and $-SO_2NH_2$;

R^2 is selected from the group consisting of -H, -OH, and $-OC_{1-4}$ alkyl;

R^3 is selected from the group consisting of $-NHCOO(CH_2)_3N(CH_3)_2$, -NHCO(4-methyl piperazinyl), $-NHSO_2(CH_2)_2N(CH_3)_2$, -OH, $-OC_{1-4}$ alkyl, -COOH and $-NHCONH(CH_2)_3CH_3$.

2. A compound according to claim 1 selected from the group consisting of: (a) 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-(3-(N, N-dimethylamino)prop-1-yloxycarbonylamino)benzyl) pyrazolo[3,4-d]pyrimidin-4-one; (b) 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-(4-methylpiperazin-1-ylcarbonylamino)benzyl)pyrazolo[3,4-d]pyrimidin-4-one; (c) 1-(2,4,6-Trichlorophenyl)-3-isopropyl-6-(4-(2-(dimethylamino)ethanesulfonamido)benzyl)pyrazolo[3,4-d]pyrimidin-4-one; (d) N-(3,5-Dichloro-4-[3-isopropyl-6-(3-methoxy-benzyl)-4-oxo-4,5-dihydro-pyrazolo[3,4-

d]pyrimidin-1-yl]-phenyl}-formamide; (e) 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-methoxybenzyl)pyrazolo[3,4-d]pyrimidin-4-one; (f) 4-{6-[4-(3-Butyl-ureido)-benzyl]-3-isopropyl-4-oxo-4,5-dihydro-pyrazolo[3,4-d]pyrimidin-1-yl}-3,5-dichloro-benzenesulfonamide; (g) 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-carboxybenzyl)pyrazolo[3,4-d]pyrimidin-4-one.

3. A compound according to claim 1 selected from the group consisting of: (a) 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-(3-(N, N-dimethylamino)prop-1-yloxycarbonylamino)benzyl) pyrazolo[3,4-d]pyrimidin-4-one; (b) 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-(4-methylpiperazin-1-ylcarbonylamino)benzyl)pyrazolo[3,4-d]pyrimidin-4-one; (c) 1-(2,4,6-Trichlorophenyl)-3-isopropyl-6-(4-(2-(dimethylamino)ethanesulfonamido)benzyl)pyrazolo[3,4-d]pyrimidin-4-one; (d) 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-carboxybenzyl)pyrazolo[3,4-d]pyrimidin-4-one.

4. A compound according to claim 1 selected from the group consisting of (a) N-{3,5-Dichloro-4-[3-isopropyl-6-(3-methoxy-benzyl)-4-oxo-4,5-dihydro-pyrazolo[3,4-d]pyrimidin-1-yl]-phenyl}-formamide; (b) 1-(2,4,6-trichlorophenyl)-3-isopropyl-6-(4-methoxybenzyl)pyrazolo[3,4-d]pyrimidin-4-one; (c) 4-{6-[4-(3-Butyl-ureido)-benzyl]-3-isopropyl-4-oxo-4,5-dihydro-pyrazolo[3,4-d]pyrimidin-1-yl}-3,5-dichloro-benzenesulfonamide.

5. A compound according to claim 1 wherein R_1 is -Cl, R_2 is -H and R_3 is -COOH.

6. A compound according to claim 1 wherein R_1 is $-SO_2NH_2$, R_2 is -H and R_3 is $-NHCONH(CH_2)_3CH_3$.

7. A compound according to claim 1 wherein R_1 is -Cl, R_2 is -H and R_3 is $-OCH_3$.

8. A compound according to claim 1 wherein R_1 is -NHCHO, R_2 is -H and R_3 is $-OCH_3$.

9. A compound according to claim 1 wherein R_1 is -Cl, R_2 is -H and R_3 is $-NHSO_2(CH_2)_2N(CH_3)_2$.

10. A compound according to claim 1 wherein R_1 is -Cl, R_2 is -H and R_3 is -NHCO(4-methyl piperazinyl).

11. A compound according to claim 1 wherein R_1 is -Cl, R_2 is -H and R_3 is $-NHCOO(CH_2)_3N(CH_3)_2$.

12. A pharmaceutical composition comprising a compound of Formula I according to claim 1 and a pharmaceutically acceptable excipient.

13. A method of inhibiting cdk activity in a patient in need of such treatment comprising the steps of administering to said patient a theraputically effective amount of a compound according to claim 1.

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(54) Title: 6-SUBSTITUTED PYRAZOLO [3,4-d] PYRIMIDIN-4-ONES USEFUL AS CYCLIN DEPENDENT KINASE INHIBITORS

(57) Abstract: This invention relates to 6-substituted pyrazolo[3,4-d]pyrimidin-4-ones useful as cyclin dependent kinase (cdk) inhibitors, pharmaceutical compositions comprising the same and methods for using these compounds for treating cancer and proliferative diseases.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/519 ; C07D 487/02
US CL : 514/258 ; 544/262, 256, 253
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/21926 (DU PONT PHARMACEUTICALS COMPANY) 20 April 2000 (20.04.2000), examples 50, 53 (page 56), example 345 (page 91), examples 5, 6 (page 93), example 5 (page 94) and claims 1-8.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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